Chemical composition and antioxidant potential of essential oils from different *Ocimum* species (Basil)

Composição química e atividade antioxidante de óleos essenciais de diferentes espécies de Ocimum (Manjericão)

DOI:10.34117/bjdv7n3-241

Recebimento dos originais: 08/02/2021
Aceitação para publicação: 01/03/2021

Celma de Oliveira Barbosa
Universidade Federal do Ceará
Padetec - Campus do Pici, Padetec, Av. Mister Hull bloco 310
Doutora em Biotecnologia pela Universidade Federal do Ceará
E-mail: celmaoliver@yahoo.com.br

Selene Maia de Morais
Universidade Estadual do Ceará
UECE
Endereço: Av. Dr. Silas Munguba, 1700 - Itaperi, Fortaleza - CE, 60714-903
Pós-doutora em Química dos Produtos Naturais pela Universidade de Aveiro, UA, Portugal
E-mail: selenemaiademorais@gmail.com

Halisson Araújo de Sousa
Universidade Federal do Ceará
Campus do Pici - Bloco 906
Mestre em bioquímica pela Universidade Federal do Ceará
E-mail: halisson.araujo@gmail.com

Vitor Carvalho Martins
Universidade Federal do Ceará
Doutorando em Química pela Universidade Federal do Ceará
Av Mister Hull, S/n - Bl 709
E-mail: vitor_martinsn1@hotmail.com

João Francisco Câmara Neto
Universidade Federal do Ceará
Doutorando em Química pela Universidade Federal do Ceará
Av Mister Hull, S/n - Bl 709
E-mail: neto-casparov@hotmail.com

Icaro Gusmão Pinto Vieira
PADETEC – Parque de Desenvolvimento Tecnológico, Universidade Federal do Ceará
Padetec - Campus do Pici, Padetec, Av. Mister Hull bloco 310
Pós- Doutorado em Recursos Naturais, Universidade Estadual do Ceará
E-mail: icarogpv@uol.com.br

Rita de Cássia Alves Pereira
Embrapa Agroindústria Tropical
Doutora em Agronomia (Fitotecnia) pela Universidade Federal de Lavras
Rua Dr[a]. Sara Mesquita, nº 2270, Bairro Planalto do Pici, CEP 60511-110, Fortaleza, CE
E-mail: rita.pereira@embrapa.br

Ana Livya Moreira Rodrigues
Universidade Estadual do Ceará
Doutoranda em Biotecnologia pela Universidade Estadual do Ceará
UECE- Endereço: Av. Dr. Silas Munguba, 1700 - Itaperi, Fortaleza - CE, 60714-903
E-mail: livya_rodrigues@hotmail.com

José Osvaldo Beserra Carioca
Universidade Federal do Ceará
Pós-doutorado em Engenharia química pela Universidade de Sttutgart, Alemanha.
Padetec - Campus do Pici, Padetec, Av. Mister Hull bloco 310
E-mail: carioca@ufc.br

ABSTRACT
Plants of the genus Ocimum are widely used in cooking, cosmetics and folk medicine, mainly antibacterial, antifungal and antioxidant properties. This article examines the chemical composition and antioxidant capacity of essential oils of Ocimum varieties. The chemical composition was analyzed by gas chromatography/mass spectrometry (GC/MS). For the evaluation of the antioxidant capacities, three different methods were used: the 2,20-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH), ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) antiradical method and β-carotene/peroxyl radicals (LOO•) systems. There was a great variability in the composition and some species had a high eugenol content: *O. tenuiflorum*>*O. campechianum*>*O. basilicum* var. ball>*O. gratissimum* >*O. basilicum* var. greek. Methyl chavicol, neral and geranial were components found in other *Ocimum* species. Better antioxidant efficiency was found in species with a higher eugenol content as *Ocimum tenuiflorum* by DPPH IC_{50} 2.31±0.02 µg.mL^{-1}, ABTS IC_{50} 2.22±0.23 µg.mL^{-1} and β-carotene/linolenic acid system IC_{50} 16.11±3.59 µg.mL^{-1}, equally statistically significant when compared to pure eugenol. Basil essential oils had higher eugenol content in five species with strong antioxidant potential, adding a differential value to the Food Industry in the production of new functional foods or biofilms for foods with functionalities of preservation or control of chronic diseases.

Keywords: basil. Ocimum. essential oil. Chemotypes. antioxidant potential.

RESUMO
As plantas do gênero Ocimum são amplamente utilizadas na culinária, cosmética e medicina popular, principalmente propriedades antibacterianas, antifúngicas e antioxidantes. O objetivo do estudo foi analisar a composição química e a capacidade antioxidante dos óleos essenciais das variedades de Ocimum. A composição química foi analisada por cromatografia gasosa/espectrometria de massas (CG/EM). A capacidade antioxidante foi realizado por três métodos diferentes: o método antiradical 2,2-difenil-1-picril-hidrazil (DPPH), ABTS (2,2′-azinobis-(3-etilbenzotiazol-6-ácido sulfônico)) e o sistema β-caroteno/ácido linoléico. Houve uma grande variabilidade na composição química e elevado teor de eugenol nessa ordem: O. tenuiflorum>O. campechianum>O.

basilicum var. bola>O. gratissimum >O. basilicum var. greco. Metil chavicol, neral e geranial foram componentes encontrados em outras espécies de Ocimum. A melhor eficiência antioxidante foi encontrada em espécies com maior teor de eugenol como *Ocimum tenuiflorum* (DPPH: CI_{50} 2,31±0,02 µg/mL, ABTS: CI_{50} 2,22±0,23 µg/mL e sistema β-caroteno/ácido linoléico: CI_{50} 16,11±3,59 µg/mL), igualmente significativo estatisticamente quando comparado ao eugenol puro. Os óleos essenciais de manjericão apresentaram majoritariamente o eugenol em cinco espécies com forte potencial antioxidante, agregando um valor diferencial à Indústria alimentícia na produção de novos alimentos funcionais ou biofilmes para alimentos com funcionalidades de preservação ou controle de doenças crônicas.

Palavras-chave: manjericão. Ocimum. óleo essencial. Quimiotipos. potencial antioxidante.

1 INTRODUCTION

The plants belonging to the basil genome or Ocimum genus of the Lamiaceae family are aromatic ones and are a rich source of essential oils-the metabolites, synthesized by plants for specific functions, using various secondary metabolic pathways.[1] All parts of basil plants were used in folk medicine for treatment of cold, coughs, as a sedative, and for eliminating toxins.[2] Also, basil is used in making flavoring and perfume, and flavor and aroma in foods.[3, 4]

It is reported in the literature that the Ocimum genus has been used in popular medicine as pharmaceutical agents because of their antimicrobial, antiemetic, antidiabetic, antifertility, antiasthmatic, antistress and anticancer activity.[5] Moreover, it is well reported that essential oils of some Ocimum species, including *Ocimum gratissimum*, *Ocimum basilicum*, *Ocimum sanctum* and *Ocimum canum* have strong antioxidant capacity.[1, 5, 6, 7]

The antioxidant activity of natural products has been widely studied in recent years in both in vitro and in vivo models, proving their significant radical scavenging properties.[8]

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus,

and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids.[9]

Some of the nutritional antioxidants will retard the oxidative process and prevent disease[9], and are capable of stabilizing or deactivating free radicals before they attack targets in biological cells.[10, 11]

Currently, essential oils (EO) of the Ocimum genus have well-known antioxidant properties, including many varieties with essential oils rich in methyl-chavicol, eugenol, citral, linalool, trans-anethole, estragol and thymol.[12, 13]

As the chemical composition (chemotype) and the biological activity of essential oils distilled from plants belonging to the same species can vary significantly, depending on the variety of cultivars environment, elevation and cultivation methods, it is interesting to compare the essential oils obtained from the different kinds of basil grown in Armenia, Asia, Africa, Europa, North American, under similar conditions, and in Brazil. Therefore, it is interesting to look for new biologically active compounds in plants of Ocimum species cultivated in Northeast Brazil that can be used as medicinal agents.

The aim of this study was to analyze the chemical composition of the essential oils of several species of Ocimum cultivated in Fortaleza city, capital of the state of Ceará, located in the Northeast of Brazil, to compare with data from the literature of plants from other places and evaluate their potential antioxidant, seeking to attribute these activities to its main constituents and trying to justify their medicinal use.

2 MATERIALS AND METHODS

2.1 PLANT AND EXTRACTION OF ESSENTIAL OILS

The plant material was cultivated and collected in the morning at a farm of Embrapa Tropical Agroindustry (Ceará), located at the Federal University of Ceará (Ceará) campus.

The *Ocimum* species were cultivated, using tilled livestock manure and daily irrigation with high solar incidence, at a medium temperature of 28 ºC. The collection was carried out in the morning (7a.m). All plants were registered in Prisco Bezerra Herbarium of the Federal University of Ceará (Table 1).

The essential oils were extracted at Natural Products Chemistry Laboratory, Ceará State University by hydrodistillation as described in the literature by A.O.A.C., 1995.[14] Table 1 shows the studied Ocimum species, genetic certification and the yield of each essential oil obtained.

2.2 GAS-CHROMATOGRAPHY/MASS SPECTRAL ANALYSIS (GC–MS)

The chemical constituents were analyzed by gas chromatography coupled to a Shimadzu QP-2010 mass spectrometer instrument. The conditions were performed with DB-1 MS column (Agilent, part Nº 122-5532) coated fused silica capillary column (30 m × 0.25 mm × 0.25 µm). The helium gas was used in constant linear velocity of 1 mL/min and the injector temperature was 250 ºC, in split mode (1:100), the detector temperature was at 250 ºC. The column temperature was programmed to 35–180 ºC at 4 ºC/min then 180– 280 ºC at 17 ºC/min, and at 280 ºC for 10 min; mass spectra: electron impact 70 eV. The injection volume of the sample was 1µL. The retention times and mass spectra were used to tentatively identify the compounds, comparing to data bank (NIST) and catalog[15].

2.3 DETERMINATION OF ANTI-FREE RADICAL ACTIVITY

2.3.1 DPPH (1,1-diphenyl- 2 – picrylhydrazyl) antiradical method

In the DPPH test, solutions of the essential oils were prepared in the following concentrations: 250, 125, 25, 12.5, 1.25, 0.25, 0.125, and 0.025 µg mL^{-1}. The negative control was a DPPH methanol solution and the positive control was prepared by mixing a standard (eugenol) to DPPH. Methanol solutions of essential oils (100 µL) was added to a test tube containing 3.9 mL of 6.5×10^{-5} mol L^{-1} DPPH methanol solution. The test was performed in triplicate and after 60 minutes in the dark, the absorbance of the mixture was measured by spectrophotometer (Genesys 10S uv-vis, Thermo Fisher Scientific, Made in China) at the wavelength of 515 nm, developed by Yepez et al., 2002.[16] The DPPH free radical inhibition was calculated by the scavenging index (SI) = (Abs DPPH–Abs Sample) x 100/Abs Sample. The inhibitory potential (%) was applied in the Origin 7.0 statistic program to calculate the 50% inhibitory concentration (IC_{50}).

2.3.2 ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) antiradical method

ABTS ethanol solution was prepared to obtain an absorbance around 0.715 at 734 nm with readings made in a spectrophotometer (Genesys 10S uv-vis, Thermo Fisher Scientific, Made in China). Several solutions of decreasing concentrations of essential oils (10000 to 5 µg mL^{-1}) were tested in triplicate, and 30 µL of these solutions were added to 3.0 mL of $ABTS^{+}$ solution and readings taken after 6 min at 734 nm, as described by Re et al., 1999.[17] The negative control was an ABTS ethanol solution and the positive control was prepared by mixing a standard (eugenol) to ABTS.

The radical scavenging activity was evaluated the percentage of inhibition of according to the following equation: IP% inhibition = (Abs ABTS–Abs Sample) x 100/Abs ABTS.

The inhibitory potential (%) was applied in the Origin 7.0 statistic program to calculate the IC_{50} (Medium inhibition concentration) by linear regression curve.

2.3.3. Determination of antioxidant capacity by co-oxidation of β-carotene/linoleic acid

Briefly, 2 mL β -carotene solution (0.2 mg mL^{-1} chloroform) was pipetted into a round-bottom flask containing 20 µL linoleic acid and 200 µL Tween 40. The mixture was evaporated at room temperature to remove chloroform. After evaporation, the mixture was added to 100 mL of distilled water saturated with oxygen. Concentrations of 200,100, 50, and 25 µg mL^{-1} of samples in methanol were prepared in test tubes and 0.2 mL aliquots were added to 5 mL of the solution of β-carotene-linoleic acid. A solution without the β-carotene-linoleic acid was prepared under the same conditions for each concentration (control solution). All the mixtures were incubated at 50 ºC for 2 h and measured in spectrophotometer (Genesys 10S uv-vis, Thermo Fisher Scientific, Made in China) at the wavelength of 470 nm, Wettasinghe & Shahidi, 1999.[18] Absorbance of the essential oils was measured immediately, the antioxidant activity was calculated and applied in Origin 7.0 to calculate the IC_{50}.

The negative control was β-carotene/linoleic acid solution and the positive control was prepared by mixing a standard (eugenol).

2.4 STATISTICAL ANALYSIS

Data were expressed as measure of central tendency and dispersion. Normality of the data was tested using the Kolmogorov-Smirnov test and the homogeneity of the data using the Levene test. For the comparison between two means, with normal and homogeneous data, the Student's test was used for independent samples, and with non-normal and non-homogeneous data, the Mann-Whitney test was used. For the comparison between three means, when the data were normal and homogeneous, the ANOVA test was used, and when not, the Kruskal-Wallis test was used. Fisher's LSD multiple-posttest was used. The data were considered significant with p values below 0.05.

3 RESULTS AND DISCUSSION

3.1 YIELDS AND CHEMICAL COMPOSITION OF ESSENTIAL OILS BY GAS-CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS

The low yields of essential oils of Ocimum species (0.03–2.00%) shown in Table 1 can be associated to a series of factors such as the genotype, plant age or plant part, extraction processes, season and collection time[19]. Regarding the results, the values of yields were bigger when compared to Sartoratto et al., 2004[20], which obtained 0.1%, and in relation to *Ocimum campechianum*, yield was within of the value found by Trevisan et al., 2006[6], 0.5 to 3.5%. The *O. basilicum* varieties varied yield of 0.03 to 0.40%, being concordant with Dìaz[21] who indicated values between 0.04 to 0.7%.

Table 1. Ocimum varieties and yield of essential oils.

Scientific name	Genetic certification*	Yields (%)
Ocimum x citriodorum	**58825**	**0.12**
Ocimum selloi	**58826**	**0.40**
Ocimum gratissimum	**59298**	**0.40**
Ocimum tenuiflorum	**49103**	**0.30**
Ocimum campechianium	**59299**	**2.00**
***Ocimum basilicum* L. (greek)**	**59300**	**0.40**
***Ocimum basilicum* L. (*cinnamon*)**	**59809**	**0.31**
***Ocimum basilicum* L. ("licorice")**	**59296**	**0.20**
***Ocimum basilicum* L. ("*Maria Bonita*")**	**58997**	**0.03**
***Ocimum basilicum* L. (white)**	**59297**	**0.30**
***Ocimum basilicum* L. (ball)**	**58858**	**0.40**

* Prisco Bezerra Herbarium

The chemical composition of essential oils of Ocimum spp. species is shown in Table 2. The essential oils presented 5 to 18 chemical components and showed distinct constituents, especially oxygenated monoterpenes.

The essential oil of *Ocimum x citriodorum* had geranial and neral as main constituents. The data on *O. x citriodorum* wasn't similar when compared to Avetisyan et al., 2017[1], identifying as predominant constituents, citral (21%) and nerol (23%).

Table 2. Main chemical constituents of *Ocimum spp.* essential oils.

Constituents	**KI exp**	**Oc**	**Os**	**Og**	**Ot**	**Ocm**
α-pinene	933	-	-	0.39	-	-
octen-3-ol <1>	970	0.08	-	-	-	0.16
Sabinene	970	-	-	0.30	-	-
β-pinene	973	-	-	1.13	-	-
6-methyl-5-hepten-2-one	977	0.34	-	-	-	-
1,8-cineole (Eucalyptol)	1023	-	4.26	22.10	-	1.01
E- β-ocimene	1042	-	0.45	-	-	-
γ-terpinene	1053	-	0.33	-	-	-
2,2-dimethyl-3,4-octadienal	1087	0.52	-	-	-	-
Linalool	1096	-	17.10	0.58	-	0.76
Trans-verbenol	1140	0.76	-	-	-	-
Camphor	1143	-	0.57	-	-	-
Cis-chrysanthenol	1160	1.28	-	-	-	-
Terpineol <delta->	1166	-	-	0.29	-	-
Terpinen-4-ol	1179	-	2.41	-	-	-
α-terpineol	1191	-	-	0.80	-	-
Methyl chavicol	1208	-	**65.82**	-	-	-
Octyl alcohol acetate	1215	-	0.35	-	-	-
Nerol	1234	2.88	-	-	-	-
Neral	1249	**36.10**	-	-	-	-
Geraniol	1261	1.55	-	-	-	-
Geranial	1281	**47.62**	-	-	-	-
Thymol	1299	-	-	0.43	-	-
Elemene <delta->	1340	-	-	-	-	0.58
Eugenol	1360	-	-	**47.03**	**81.91**	**68.74**
Neryl acetate	1368	0.71	-	-	-	-
α-copaene	1381	-	-	0.31	-	-
β-elemene	1394	-	-	0.75	6.54	4.40
Methyl-eugenol	1408	-	-	-	-	1.52
α-cis-bergamotene	1420	-	3.95	-	-	-
E-caryophyllene	1426	1.66	-	7.09	10.05	7.65
α-trans-bergamotene	1437	0.67	-	-	-	-
α-humulene	1455	0.70	0.52	1.04	0.59	1.67
Allo-aromadendrene	1460	-	-	-	-	0.28
Germacrene D	1480	0.52	0.36	1.67	-	0.34

Constituents	KI exp	Oc	Os	Og	Ot	Ocm
β-selinene	1485	1.15	-	11.12	-	1.16
α-selinene	1493	0.81	-	3.20	-	-
Bicyclogermacrene	1494	-	-	-	-	4.95
α-bulnesene	1503	-	-	-	0.91	-
γ-cadinene	1510	-	0.94	-	-	-
7-epi-α-selinene	1511	-	-	0.87	-	-
β-sesquifelandrene	1520	-	-	-	-	1.45
α-Z-bisabolene	1534	1.57	-	-	-	-
Elemicin	1547	-	-	-	-	4.16
Spathulenol	1570	-	-	-	-	0.35
Caryophyllene oxide	1571	1.08	-	0.91	-	0.82
Epi-α-cadinol	1621	-	2.47	-	-	-
% Total identified	-	100.00	99.27	100.00	100.00	99.95

Oc = Ocimum x citriodorum, Os = Ocimum selloi, Og = Ocimum gratissimum (Alfavaca), Ot = Ocimum tenuiflorum, Ocm = *Ocimum campechianium* (syn O. *micranthum*)

The essential oil of *O. selloi* showed variability with 65.82% methyl chavicol, in disagreement with other researchers who identified 52.20% anetole and 16.80% linalool[22], and a similar value of methyl chavicol (55.30%) was reported by Padilha-Paula et al., 2003.[23]

Eugenol was identified in the essential oils of *O. gratissimum* essential oils (see Table 2), similarly to studies with Viçosa plantations (Minas Gerais/Brazil)[12] and the genotypes of Goiás (Brazil)[24].

The *O. tenuiflorum* essential oil also had eugenol as its main constituent (see Table 2), showing a higher percentage compared to the research by Kothari et al., 2004, who worked with the same species grown in India and obtained between 4.36% and 8.48% of eugenol and methyl eugenol, respectively. [25] It is interesting to note that *O. tenuiflorum* had a similar eugenol content compared to *Syzygium aromaticum* (L.) (clove) (82.47%) and *Pimenta dioica* (Jamaican pepper) (82.56%), Oliveira et al., 2009[26], which are spices used in the Food Industry.

The essential oil of *O. campechianum* had few studies about its chemistry, showing a higher content of eugenol in leaves grown in Amazon (Brazil) with 46.55% eugenol[27] and 64.80% eugenol in plants from Ceara (Brazil) by Vieira et al.[22]

The chemical composition of essential oils of *Ocimum basilicum* varieties is shown in Table 3. Essential oils from *O. basilicum* presented 6 to 24 constituents with prevalence of oxygenated monoterpenes. The essential oil of *O. basilicum* var. greek a palla had

eugenol, linalool, 1,8-cineole, epi-α-cadinol, and α-cis-bergamotene as main constituents. This is the first study of the chemical composition of this oil.

Table 3. Chemical constituents of *Ocimum basilicum* L. and its varieties essential oils.

Constituents	**KI exp**	**Obg**	**Obc**	**Obl**	**Obmb**	**Obw**	**Obb**
α-pinene	933	0.30	-	-	-	-	-
Sabinene	970	0.38	-	-	-	-	-
β-pinene	971	0.75	-	-	-	-	-
Mircene	985	0.57	-	-	-	-	-
1,8-cineole	1018	11.46	3.55	3.36	3.63	2.96	1.07
Z-β-ocimene	1040	0.62	-	0.79	-	-	-
E-β-ocimene	1041	-	0.89	-	-	-	2.25
Linalool	1095	**23.54**	-	-	**70.58**	**17.08**	**10.96**
Camphor	1140	1.59	0.63	0.57	-	0.44	0.55
Borneol	1160	-	-	-	-	-	0.46
Terpinen-4-ol	1174	-	-	-	-	2.12	2.99
α-terpineol	1190	1.77	-	-	0.75	-	0.41
Methyl-chavicol	1190	-	**87.47**	**92.48**	-	**67.85**	0.39
Octyl alcohol acetate	1216	0.62	-	-	-	-	0.31
Neral	1243	-	0.99	-	-	0.79	-
Isobornyl formate	1286	-	-	-	-	-	1.12
Geraniol	1260	-	-	-	18.01	-	-
Geranial	1274	-	1.30	-	-	0.98	-
Bornyl acetate	1293	1.01	-	-	-	-	-
Eugenol	1369	**42.15**	-	-	-	-	**56.53**
Geranyl acetate	1384	-	-	-	2.51	-	-
β-elemene	1396	1.36	-	-	-	0.45	1.38
Methyl-eugenol	1405	-	-	-	-	-	0.45
α-cis-bergamotene	1437	3.24	0.79	-	2.12	-	9.27
E- Caryophyllene	1424	-	-	-	-	0.48	-
α-trans-bergamotene	1439	-	-	-	-	0.73	-
α-guaiene	1440	0.38	-	-	-	-	0.40
α-humulene	1455	0.39	0.74	-	-	0.61	-
Germacrene D	1480	0.94	-	-	-	0.72	0.91
Bicyclogermacrene	1498	0.30	-	-	-	-	-
α-bulnesene	1503	1.03	-	-	-	-	0.99
γ-cadinene	1509	1.13	0.93	0.71	0.71	1.19	2.08
Eugenol-acetate	1524	0.83	-	-	-	-	-
Spathulenol	1569	0.41	-	-	-	0.53	0.47
1,10-di-epi-cubenol	1603	0.56	-	-	-	-	-
1-epi-cubenol	1600	-	-	-	-	-	0.55
Epi-α-cadinol	1622	4.67	2.72	2.08	1.69	3.08	4.46
% Total identified	-	100.00	100.00	99.99	100.00	100.00	96.15

Obg = *Ocimum basilicum* var greek palla, Obc = *Ocimum basilicum* var cinnamon, Obl = *Ocimum basilicum* var licorice, Obmb = *Ocimum basilicum* var Maria Bonita, Obw = *Ocimum basilicum* var white, Obb = *Ocimum basilicum var ball*

The essential oils of *O. basilicum* known as cinnamon, "licorice" and white have shown methyl chavicol as the main constituent (see Table 3). A similar content for methyl chavicol as the main constituent in *Ocimum basilicum* has been reported in plants from Turkey (78.02%)[28] and leaves grown in India (39.30%).[29]

Linalool was the main chemotype in *O. basilicum* variety "Maria bonita" (see Table 3). According to this study, Blank et al., 2007, analyzed the same variety of origin in the EUA with 78.12% linalool.[30] Another similar study, Veloso et al. (2014) determined 36.32% of linalool in plants in Tocantins (Brazil).[13]

The *O. basilicum* var. ball had a higher content of eugenol and linalool, showing significant differences when compared to Joshi, 2014, who identified chemotypes of methyl eugenol and methyl chavicol in plants from India (*O. basilicum*).[31]

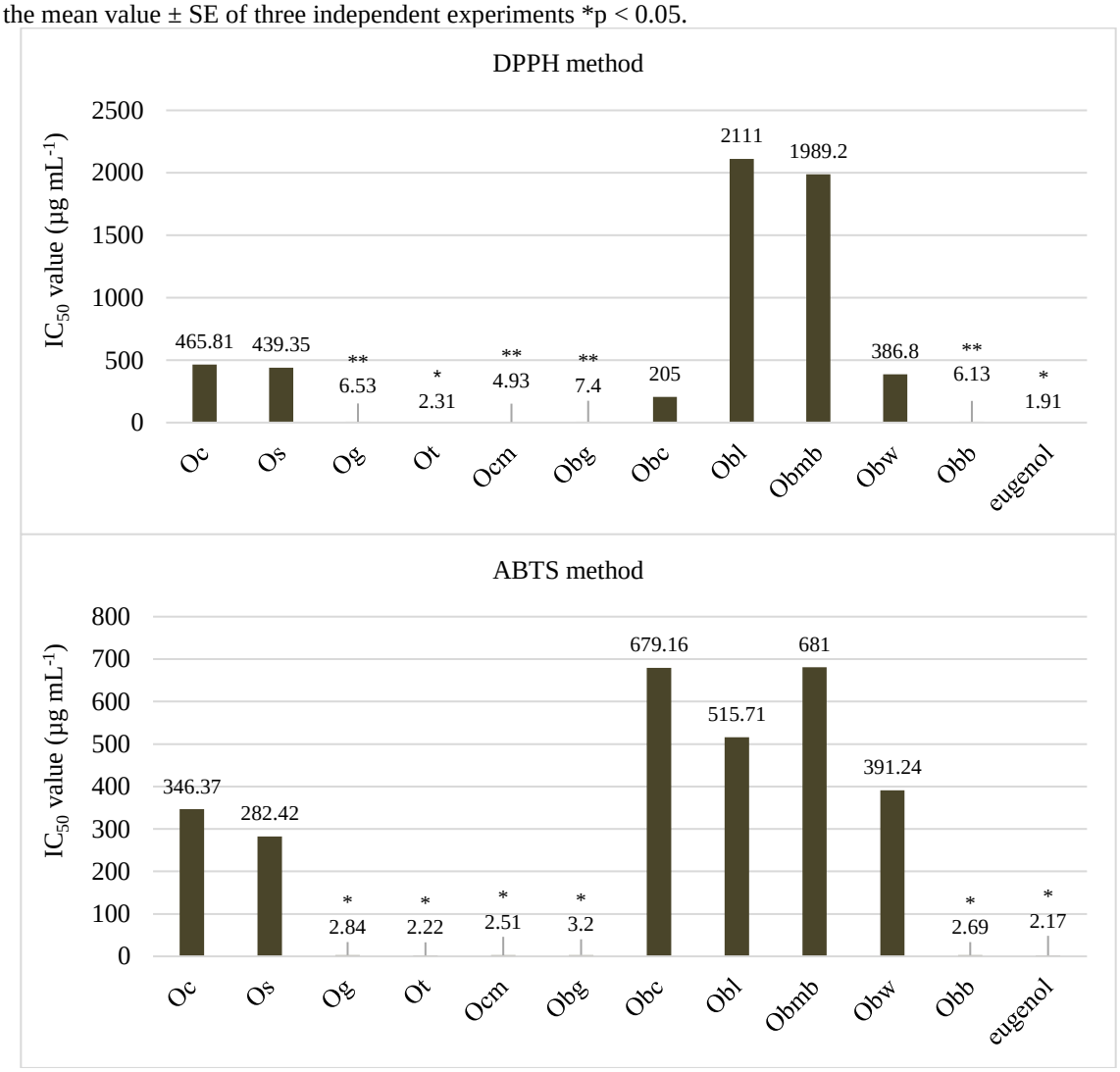
3.2 ANTIOXIDANT ACTIVITY

Figure 1 shows the antioxidant potential of *Ocimum* varieties. Statistical analyzes showed little similarity between the activities of the oils. There was a strong relationship between the composition of essential oils and antioxidant activity by the DPPH and ABTS methods, while in the method with ABTS radical this relationship was more prominent.

The result showed the best DPPH antiradical action for the essential oil of *Ocimum tenuiflorum*, with no statistical difference when compared to pure eugenol ($p>0.05$). There was good antioxidant activity in the species *Ocimum gratissimum*, *Ocimum campechianum*, *Ocimum basilicum* var. greek and *Ocimum basilicum* var. ball, no statistical difference was obtained between these samples ($p>0.05$).

Bunarathep et al., 2007,[7] reported that *O. gratissimum* was more active when compared to *O. sanctum* (known as *O. tenuiflorum*), *O. canum* and *O. basilicum* with IC_{50} values of 30.20, 767.82, 8434.19 and 47057.45 μg mL^{-1}, respectively. In relation to Figure 1, the IC_{50} values showed excellent antioxidant potential when compared to Bunarathep et al.[7]. Similar data were found by Trevisan et al., 2006, who studied the antioxidant capacity of five species of *Ocimum*, and the best result was shown for the essential oil of *O. tenuiflorum*.[6]

Figure 1: IC_{50} values of Ocimum essential oils and eugenol by DPPH and ABTS methods. The data represent the mean value ± SE of three independent experiments *p < 0.05.



Oc = Ocimum x citriodorum, Os = Ocimum selloi, Og = Ocimum gratissimum (Alfavaca), Ot = Ocimum tenuiflorum, Ocm = *Ocimum campechianium* (syn O. *micranthum*), Obg = *Ocimum basilicum* var greek palla, Obc = *Ocimum basilicum* var cinnamon, Obl = *Ocimum basilicum* var licorice, Obmb = *Ocimum basilicum* var Maria Bonita, Obw = *Ocimum basilicum* var white, Obb = *Ocimum basilicum var ball*

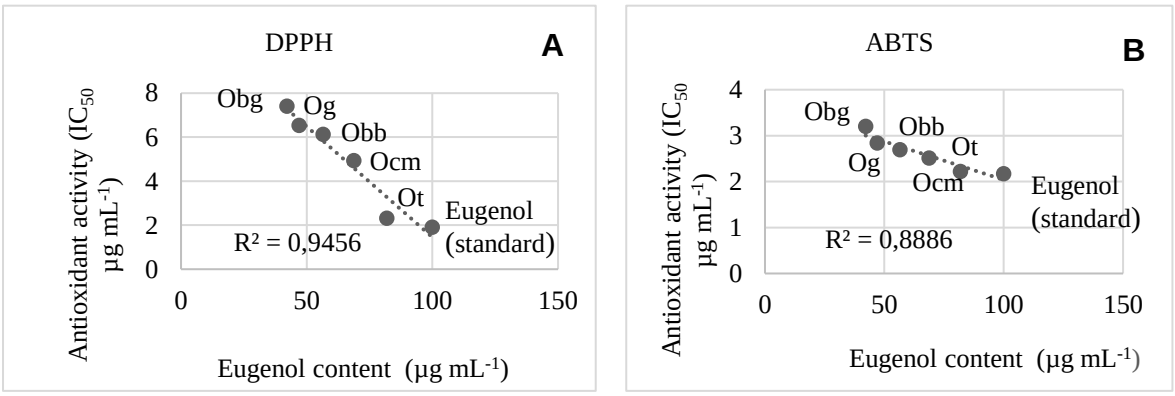
Essential oils are a complex mixture of various compounds with different functional groups and polarity. The antioxidant effect of an essential oil cannot be attributed to one or few constituents, but to its general structural complexity, and compounds sometimes with lower concentrations can contribute significantly to the oil activity.[32]

The essential oils of *O. tenuiflorum*, *O. gratissimum*, *O. campechianum* and *O. basilicum* (greek and ball varieties) showed similar activity when compared to the eugenol standard by the ABTS antiradical method (Figure 1), with no statistical difference (p>0,05). Then, there was a positive relation between the eugenol content in the samples and the antioxidant activity measured by the DPPH radical (hydrophilic medium) and ABTS

(hydrophilic and lipophilic medium)[33], showing a regression of approximately 0.9 (see Figure 2). The antioxidant capacity of eugenol has been reported in many studies.[12, 34]

According to Tomaino et al.[35] and Trevisan et al.[6], this activity was positively correlated in essential oils with a high proportion of eugenol, while a strong negative correlation was observed when other major compounds were identified (see Figure 2). It is worth mentioning that eugenol is the active ingredient of cloves (85-92%), of plants of the genus Ocimum, of cinnamon and nutmeg, having capacity explained by the structural characteristic of the phenolic group that strongly steals free radicals[6, 12] and slows down the autoxidation of linoleic acid.[36]

Figure 2. A) Correlation between eugenol content and antioxidant activity by DPPH method. B) Correlation between eugenol content and antioxidant activity by ABTS method.

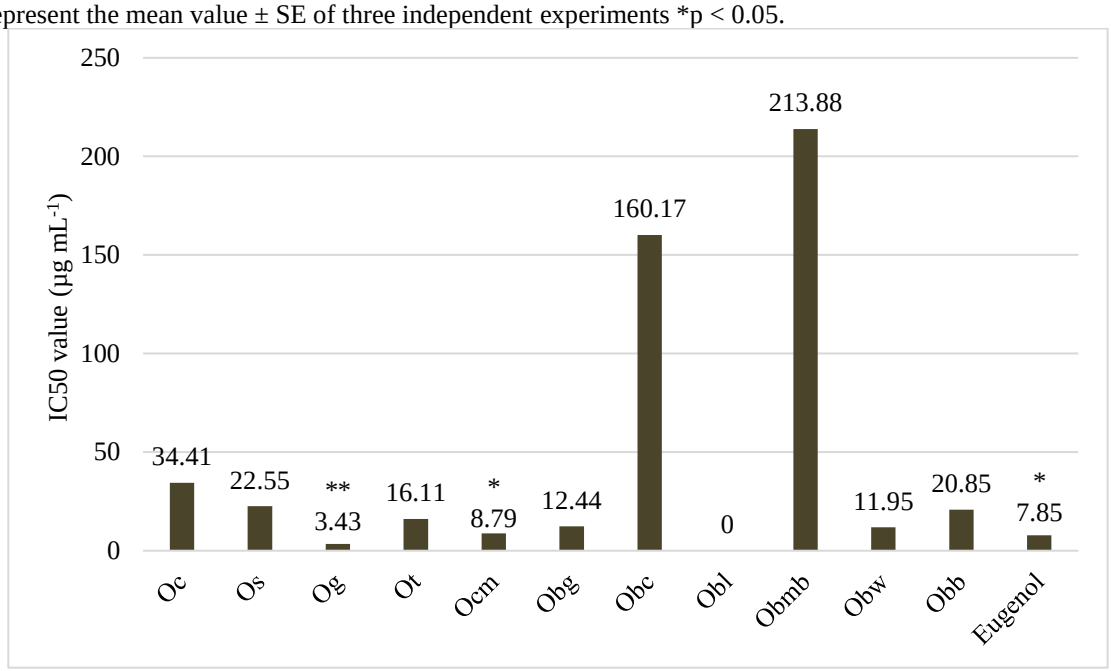


Obg = *Ocimum basilicum* var greck palla, Og = *Ocimum gratissimum*, Obb = *Ocimum basilicum var ball*, Ocm = *Ocimum campechianum*, Ot = *Ocimum tenuiflorum.*

The properties already known of eugenol are antioxidant functions[37,38], anticarminatives, antispasmodics, antiseptics and antimicrobials, also very well known as anesthetic and analgesic in dentistry.[22]

Figure 3 shows the antioxidant potential of *Ocimum* varieties by the beta-carotene/linoleic acid system.

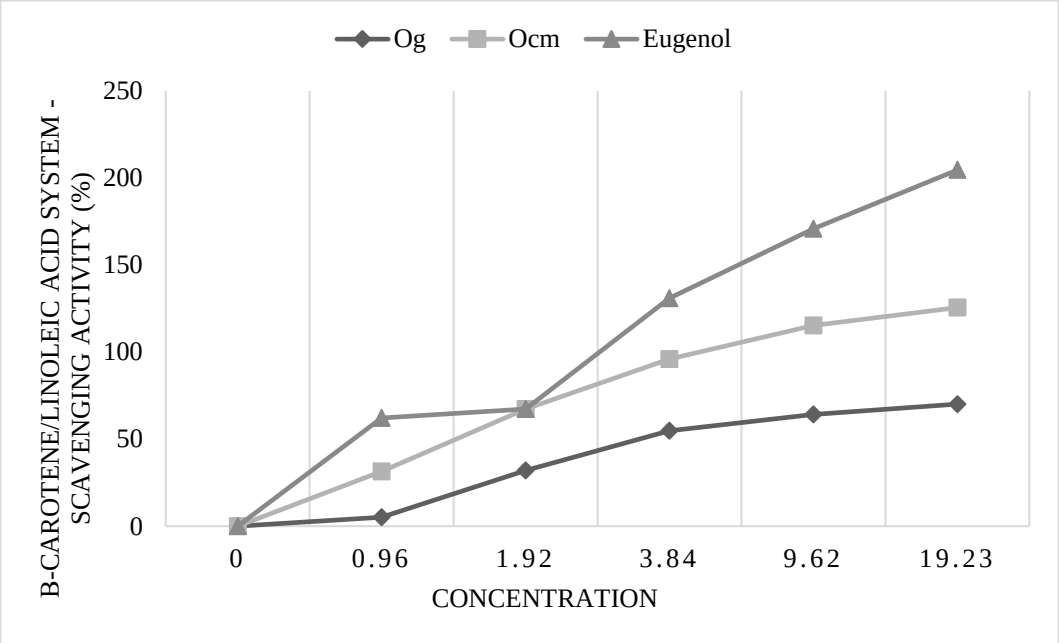
Figure 3. IC_{50} values of Ocimum essential oils and eugenol by β-carotene/linoleic acid system. The data represent the mean value ± SE of three independent experiments *p < 0.05.



Oc = Ocimum x citriodorum, Os = Ocimum selloi, Og = Ocimum gratissimum (Alfavaca), Ot = Ocimum tenuiflorum, Com = *Ocimum campechianium* (syn O. *micranthum*), Obg = *Ocimum basilicum* var greek palla, Obc = *Ocimum basilicum* var cinnamon, Obl = *Ocimum basilicum* var licorice, Obmb = *Ocimum basilicum* var Maria Bonita, Obw = *Ocimum basilicum* var white, Obb = *Ocimum basilicum var ball*

The antioxidant activity determined by the beta-carotene/linoleic acid system is a method that estimates the inhibition of the formation of free radicals, based on the activity of a sample or compound to protect a lipid substrate from oxidation[39], being able to estimate the antioxidant capacity *in vivo* and the stability of the sample tested in fats. This result was able to identify *O. gratissimum* as the species with strong antioxidant capacity and better scavenging activity (%) in 19.23 µg mL^{-1} (Figure 3 and 4), even more active than pure eugenol, differing statistically ($p < 0.05$). It is worth mentioning that this result is interesting, showing that this essential oil showed the ability to inhibit the formation of free radicals due to the oxidation promoting factors used in this in vitro test: oxygen and high temperatures, possibly promoting free radical scavenging *in vivo* and food stability. The essential oil of *O. campechianum* showed good antioxidant capacity by the beta-carotene/linoleic acid method, with no statistical difference in IC_{50} value and better scavenging activity (%) in 19.25 µg mL^{-1} when compared to the eugenol standard (Figure 3 and 4). Statistically, *O. basilicum* (greek and white varieties) and *O. tenuiflorum* were equally significant with moderate antioxidant capacity (see Figure 3).

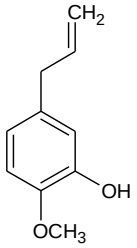
Figure 4. Antioxidant capacity of *Ocimum gratissimum* and *Ocimum campechianum* compared to the eugenol standard in different concentrations (μg mL^{-1}) by β-carotene/linoleic system.



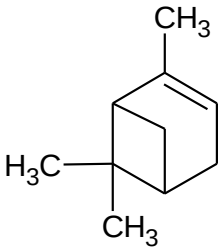
The presence of phenylpropanoids, which have the methoxy (CH_3O) and hydroxyl (OH) groups in their structure, is the main component of this activity. These include eugenol (1) and methyl-eugenol (2). The presence of methoxy (CH_3O) and hydroxyl (OH) groups in the structure of the phenylpropanoids (Figure 5) provides the compound's ability to donate protons, contributing to its antioxidant activity. On the other hand, α-pinene (3), β-pinene (4), γ-terpinene (5) and p-cymene (6) mentioned by Zhang et al., 2019[40]; Lima & Cardoso, 2007[41], revealing low activity, but it can increase activity and act synergistically with other compounds (Figure 5).

It is evident that the biological potential of eugenol is very promising and that modifications in its structure can optimize various biological activities, in order to improve the interactions with a certain biological target, contributing positively to human health.[42]

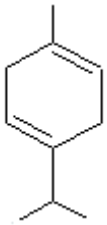
Figure 5. Representation of chemical structures of eugenol (1), methyl-eugenol (2), α-pinene (3), β-pinene (4), γ-terpinene (5) and p-cymene (6).



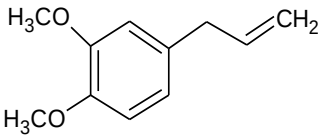
Eugenol (1)



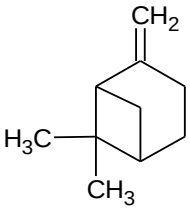
α-pinene (3)



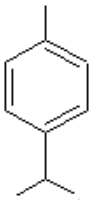
γ - terpinene (5)



Methyl -eugenol (2)



β-pinene (4)



***p*-cymene (6)**

4 CONCLUSION

The composition of the essential oils from eleven plants of the genus Ocimum evaluated showed a predominance of phenylpropanoids, mainly the compounds: eugenol and methyl-eugenol. The antioxidant activity in Ocimum species is related to a higher eugenol content mainly in *Ocimum tenuiflorum*, *Ocimum gratissimum*, *Ocimum campechianum* and *Ocimum basilicum* (greek and ball varieties).

Then, the presence of eugenol as the most active component in essential oils from Ocimum, in addition to minor active constituents, can be associated with the functional properties of Ocimum species and encourages the discovery of new natural antioxidant and possible antitumor activity with biotechnological possibilities as natural food preservatives or clinical applications.

ACKNOWLEDGEMENTS

We are thankful for the financial support of FUNCAP (Ceará State Research Funding) and Education Secretary of the Ceará State. The authors thank to Department of Organic and

Inorganic Chemistry, Federal University of Ceará, for the analysis of essential oils by GC/MS.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

REFERENCES

1 - Avetisyan, A., Markosian, A., Petrosyan, M., Sahakyan, N., Babayan, A., Aloyan, S., Trchounian, A, 2017. Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars. *BMC Compl. and Altern. Med*. 17, 1-8. http://doi.org/10.1186/s12906-017-1587-5.

2 - Sharafati-Chaleshtori, R., Rokni, N., Rafieian-Kopaei, M.; Drees, F.; Salehi, E., 2015. Antioxidant and antibacterial activity of basil (*Ocimum basilicum* L.) essential oil in beef burger. *J. Agric. Sci. Technol*. 17, 817-826.

3 - Telci, I., Bayramb, E., Yılmaza, G., Avcı, B., 2006. Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). *Biochem. Syst. Ecol.***,** 34, 489–497. https://doi.org/10.1016/j.bse.2006.01.009.

4 - Elsherbiny, E.A., El-Khateeb, A.Y., Azzaz, N.A., 2016. Chemical composition and fungicidal effects of *Ocimum basilicum* essential oil on bipolaris and cochliobolus species. J. Agric. Sci. Technol. 18, 1143-1152.

5 - Selvi, M. T., Thirugnanasampandan, R., Sundarammal, S., 2015. Antioxidant and cytotoxic activities of essential oil of Ocimum canum Sims. from India. *J. Saudi Chem. Soc*. 19, 97–100. https://doi.org/10.1016/j.jscs.2011.12.026.

6 - Trevisan, M.T.S., Vasconcelos Silva, M.G., Pfundstein, B., Spiegelhalder, B., Owen, R.W., 2006. Characterization of the volatile pattern and antioxidant capacity of essential oils from different species of the genus Ocimum. *J. Agric. Food Chem*. 54, 4378-4382. https://doi.org/10.1021/jf060181+.

7 - Bunarathep, S., Palanuvez, C., Ruangrungsi, N., 2007. Chemical composition and antioxidative activities of essential oils from four *Ocimum* species endemic to Thailand. *J. Health Res*. 21, 201–206.

8 - Baj, T., Baryluk, A., Sienawaska, E. 2018. Application of mixture design for optimum antioxidant activity of mixtures of essential oils from Ocimum basilicum L., Origanum majorana L. and Rosmarinus officinalis L. *Industrial Crops and Products* 115, 52-61. https://doi.org/10.1016/j.indcrop.2018.02.006

9 - Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*. 4, 118–126. https://doi.org/10.4103/0973-7847.70902.

10 - Atoui, A., Mansouria, A., Boskoub, G., Kefal, P., 2005. Tea and herbal infusions: their antioxidant activity and phenolic profile. *Food Chem*. 89, 27–36. https://doi.org/10.1016/j.foodchem.2004.01.075.

11 - Barros, A.S., Morais, S.M., Ferreira, P.A.T., Vieira, I.G.P., Craveiro, A.A., Fontenelle, R.O.S., Menezes, J.E.S.A., Silva, F.W.F., Sousa, H.A., 2015. Chemical composition and functional properties of essential oils from *Mentha* species. *Ind. Crops and Prod*. 76, 557–564. https://doi.org/10.1016/j.indcrop.2015.07.004.

12 – Pereira, C.A.M., Maia, J.F., 2007. Estudo da atividade antioxidante do extrato e do óleo essencial obtidos das folhas de alfavaca (*Ocimum gratissimum* L.). *Cienc. Tecnol. Alim*. 27, 624-632.

13 – Veloso, R.A.; Castro, H.G.; Barbosa, L.C.A.; Cardoso, D.P.; Chagas Júnior, A.F.; Scheidt, G.N., 2014. Teor e composição do óleo essencial de quatro acessos e duas cultivares de manjericão (*Ocimum basilicum* L.). *Rev. Bras. Plantas Med*. 16, 364-371. https://doi.org/10.1590/1983-084X/12_180.

14 – Association of Official Analytical - AOAC, 1995. Official methods of analysis. 16thed. AOAC International: Gaithersburg, MD.

15 – Adams, R. P., 2017. Identification of essential oil components by Gas Chromatography/Mass Spectrometry, 4.1th Edition, Allured Publishing Corporation: USA.

16 - Yepez, B., Espinosa, M., Lopez, S., Bolanos, G., 2002. Producing antioxidant fractions from herbaceous matrices by super-critical fluid extraction. *Fluid Phase Equil*. 194, 879–884. https://doi.org/10.1016/S0378-3812(01)00707-5.

17 – Re, R., Pellegrine, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cátion decolorization assay. *Free rad. biol. med*. 26, 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

18 – Wettasinghe, M., Shahidi, F., 1999. Evening primrose meal: a source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. *J. Agric. and Food Chem*. 47, 1801-1812, 1999. https://doi.org/10.1021/jf9810416.

19 – Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods - a review. Internat. *J. Food Microbiol*. 94, 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022.

20 - Sartoratto, A., Machado, A.L.M., Delarmelina, C., Figueira, G.M., Duarte, M.C.T., Rehder, V.L.G., 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian J. Microbiol*., 35, p. 275-80. http://dx.doi.org/10.1590/S1517-83822004000300001.

21 – Díaz, P., 2010. Efecto del tiempo de secado y de la variedad en las características físico-químicas de la albahaca (*Ocimun basilicum*) seca. Tesis en Ingeniería y Tecnología de Alimentos. Universidad Zamorano. Honduras. 18p.

22 – Vieira, P.R.N., Morais, S.M., Bezerra, F.H.Q., Ferreira, P.A.T., Oliveira, I.R., Silva, M.G.V., 2014. Chemical composition and antifungal activity of essential oils from *Ocimum* species. *Ind. Crops Prod*. 55, 267–271. https://doi.org/10.1016/j.indcrop.2014.02.032.

23 – Padilha-Paula, J., Gomes-Carneiro, M.R., Paumgartten, F.J., 2003. Chemical composition, toxicity and mosquito repellency of *Ocimum selloi* oil. *J. Ethnopharmac*. 88, 253-60. https://doi.org/10.1016/S0378-8741(03)00233-2.

24 – Vieira, R.F., Simon, J.E., 2000. Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. *Economic Bot*. 54, 207-216.

25 – Kothari, S.K., Bhattacharya, A.K., Ramesh, S., 2004. Essential oil yield and quality of methyl eugenol rich *Ocimum tenuiflorum* L. (syn. *O. sanctum* L.) grown in south India as influenced by method of harvest. *J. Chromat. A*. 1054, 67–72. https://doi.org/10.1016/j.chroma.2004.03.019.

26 – Oliveira, R.A., Reis, T.V., Sacramento, C.K., Duarte, L.P., Oliveira, F.F., 2009. Constituintes químicos voláteis de especiarias ricas em eugenol. *Rev. Bras. Farmacog*. 19, 771-775. http://dx.doi.org/10.1590/S0102-695X2009000500020.

27 – Sacchetti, G., Medici, A., Maietti, S., Radice, M., Muzzoli, M., Manfredini, S., Braccioli, E., Bruni, R., 2004. Composition and functional properties of the essential oil of amazonian basil, *Ocimum micranthum* Willd., Labiatae in comparison with commercial essential oils. *J. Agric. and Food Chem*. 52, 3486-91. https://pubs.acs.org/doi/10.1021/jf035145e.

28 – Özcam, M., ChalchaT, J.C., 2002. Essential oil composition of *Ocimum basilicum* L. and *Ocimum minimum* L. in Turkey. *Czech J. Food Sci*. 20, 223-228.

29 – Zheljazkov, V.D., Cantrell, C.L., Evans, W.B., Ebelhar, M.W., Coker, C., 2008. Yield and composition of *Ocimum basilicum* L. and *Ocimum sanctum* L. grown at four locations. *Hortsc*. 43, 737–741. https://doi.org/10.21273/HORTSCI.43.3.737.

30 - Blank, A.F., Souza, E.M., Arrigoni-Blank, M.F., Paula, J.W.A., ALVES, P.B.A., 2007. Maria Bonita: cultivar de manjericão tipo linalol. *Pesq. Agropec. Brasil*. 42, 1811-1813. https://doi.org/10.1590/S0100-204X2007001200020.

31 – Joshi, R.K., 2014. Chemical composition and antimicrobial activity of the essential oil of *Ocimum basilicum* L. (sweet basil) from Western Ghats of North West Karnataka, India. *Ancient Science Life* 33, 151–6. https://doi.org/10.4103/0257-7941.144618.

32 – Miranda, C. A. S. F., Cardoso, M. G., Batista, L. R., Rodrigues, L. M. A., Figueiredo, A. C. S., 2016. Óleos essenciais de folhas de diversas espécies: propriedades antioxidantes e antibacterianas no crescimento espécies patogênicas. *Revista de Ciência Agronômica* 47, 213-220.

33 – Kuskoski, E.M., Asuero, A.G., Troncoso, A. M., Mancini Filho, J., Fett, R., 2005. Aplicatíon de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. *Ciência e Tecnologia de Alimentos* 25, 726-732.

34 – Santana, A. C. M., Uetenabaro, A. P. T., Brito e Silva, T. M., Costa, L. C. B., Oliveira, R. A., 2016. Storage conditions of Ocimum gratissimum L. leaves influence the quality of essential oil. *Journal of Essential Oil Research*, 1-8.

35 – Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., Pasquale, A., 2005. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food Chemistry* 89, 549–554.

36 – Stoilova, I., Bailb, S., Buchbauer, G., Krastanov, A., Stoyanovac, A., Schmidtd, E., Jirovetz, L., 2008. Chemical composition, olfactory evaluation and antioxidant effects of an essential oil of *Thymus vulgaris* L. from Germany. *Natural Product Communications* 3, 1047-1050.

37 – Dorman, H.J.D., Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Let. Appl. Bacteriol*. 88, 308-316.

38 – Politeo, O., Jukic, M., Milos, M., 2007. Chemical composition and antioxidant capacity of free volatile aglycones from basil (Ocimum basilicum L.) compared with its essential oil. *Food Chemistry* 101, 379-385.

39 – Duarte-Almeida, J. M., Santos, R. J., Genovese, M. I., Lajolo, F. M., 2006. Avaliação da atividade antioxidante utilizando sistema β-caroteno/ácido linoléico e método de seqüestro de radicais DPPH. *Ciência & Tecnologia de Alimentos* 26, 446-452.

40 – Zhang, N., Yao, L. 2019. Anxiolytic effect of essential oils and their constituents: a review. *J Agric Food Chem.* 67, 13790-808.

41 – Lima, R. K.; Cardoso, M. G. 2007. Família Lamiaceae: importantes óleos essenciais com ação biológica e antioxidante. *Revista Fitos* 3, 14-24.

42 - Medeiros, H. I. R., Silva, B. B. M., Aguiar, C. E. R., Brito, T. A. M., Ferreira, F. E. S., Fernandes, N. D., Moura, E. P., Medeiros Jr., F. C., 2020. Síntese, caracterização e bioensaio toxicológico do 2-(4-Alil-2-Metoxifenoxi) acetato de etila. *Brazilian Journal of Development* 9, p. 66089-66098.